Fungal Endophytes Associated With the Actinorhizal Plant Species, Elaeagnus Latifolia L. (Elaeagnaceae) And Evaluating Their Antagonistic Potential Against Grey Blight Disease In Tea [Camellia Sinensis (L.) O. Kuntze]: A Novel Study From North-East India

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Research Article

Keywords: Actinorhizal plant species, Antifungal potential, Endophytes, Elaeagnus latifolia L., Shannon and Simpson index value, Poisoned food technique.

Posted Date: March 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-260294/v1

Fungal Endophytes Associated With The Actinorhizal Plant Species, *Elaeagnus Latifolia* L. (*Elaeagnaceae*) And Evaluating Their Antagonistic Potential Against Grey Blight Disease In Tea [*Camellia Sinensis* (L.) O. Kuntze]: A Novel Study From North-East India

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Declarations

Funding

Not applicable.

Conflicts of interest/competing interests

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author.

Code availability (software application or custom code)

Not applicable

Ethics approval

Not applicable.

Consent to participate

Not applicable

Consent for publication

Not applicable

Acknowledgements

The authors are thankful to the Director, Rain Forest Research Institute, Jorhat, Assam, India and the Director, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat, Assam, India for providing the necessary facilities to undertake the study.

Abstract

The present investigation is a novel approach of exploring the endophytic fungal diversity of *Elaeagnus latifolia* L., an actinorhizal plant species of North-east India and evaluation of its biocontrol potential against *Pestalotiopsis theae*, the causal agent of grey blight disease in tea. A total of 17 endophytic fungal species belonging to 12 families and 03 orders were isolated from various parts of *E. latifolia* L. Isolates were identified based on colony morphology, spore and fruiting bodies using microscopical tools and techniques. *Nigrospora* sp. showed highest species density (0.5) among all the isolates. Isolation frequency was maximum (67%) in case of *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum* and *Rhizopus* sp. More fungal isolates were obtained from root and stem (47% each) as compared to leaves (29%). Species richness and diversity indices was maximum (15.0) in stem and minimum (9.0) in leaves. Highest Shannon and Simpson diversity index was in stem (2.02 and 0.860 respectively) followed by root (1.979 and 0.847 respectively) and least in leaves (1.494 and 0.75 respectively). Among the isolates tested for plant growth promoting parameters, EF09 (*Fusarium* sp.) showed positive response for all the tested parameters. The isolate, EF09 also showed maximum antifungal potential (up to 87.1%) against *P. theae* in poisoned food technique. Enumeration of endophytic fungal diversity of *E. latifolia* L. holds promises as the putative strains may lead to the isolation of novel bioactive components for use in industry, medicine and agriculture.

Keywords: Actinorhizal plant species, Antifungal potential, Endophytes, *Elaeagnus latifolia* L., Shannon and Simpson index value, Poisoned food technique.

Introduction

Endophytes are microorganisms inhabiting healthy plant tissues for all or part of their life cycle without causing apparent detrimental symptoms to the host (Petrini 1991). Endophytism is symptomless associations of other living organisms that grow within the living plant tissues. According to Stone et al. (2000) fungi are the most commonly studied endophytes, so far reported based on their occurrence. A wide range of plants have now been recognized to colonize with endophytes. Plants colonized by endophytic fungi are known to grow faster than non-colonized ones (Cheplick et al. 1989) and thereby suggest a long standing close and mutually beneficial interaction (Shukla et al. 2012). Zhang et al. (2006) reported that endophytic fungi harboured in medicinal plants makes the host to adapt at extreme environmental condition. The fungal communities live inside the healthy

plants and thereby play vital roles in increasing the absorption of soil nutrients and maintenance of biogeochemical cycles (Krishnamurthy et al. 2008).

Because of their immense potentiality in conferring plants the ability to adapt in stress and production of compounds with pharmaceutical importance, this group of fungi become an important component of beneficial microflora. Interest has been exaggerated on the bioactive compounds of endophytic fungi and their possible role in producing pharmacologically active substances with unique properties like anticancer, antidiabetic, insecticidal and immunosuppressives (Strobel et al. 2004) besides producing factors of plant growth, toxins and enzymes. Plants may benefit indirectly from endophytes by increased resistance to pathogens or stress (Saikkonen et al. 1998). Investigation on endophytic microbes on different crops shows positive results such as plant growth promoting activities and antagonistic effect on pest and disease control. Fungal endophytes including *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium* spp., and *Rhizopus stolonifer* isolated from diverse kinds of plants were reported for their ability to produce different growth promoting hormones (Hassan, 2002; Waqas et al. 2012) and mineral solubilizing activities including phosphate, zinc and potassium. A vast majority of plants are recognized as repository of fungal endophytes with novel metabolites of pharmaceutical and agricultural importance (Strobel et al. 2004). It has been reported that even though most of them are host specific, certain groups of endophytic fungi possess a greater occurrence in some plants indicating their host preferences (Manganyi and Ateba 2020).

Elaeagnus latifolia L. belongs to family *Elaeagnaceae,* locally known as *Soh-shang* in Khasi hills of Meghalaya and *Mirika Tenga* in Assam, is a large evergreen spreading type woody shrub with rusty-shiny scales that are often thorny. The flowers are hermaphrodite and are pollinated by bees. The indigenous people have found many uses of *Soh-shang* fruit besides enjoying it as fresh fruit (Seal 2011). Fruits are eaten raw and could be utilized for making jam, jelly and refreshing drink. The fruit is considered to be a very rich source of vitamins, essential fatty acids, minerals and other bioactive compounds. However, very little attempts have been made to enumerate the assemblance of endophytic microbial diversity associated with this economically important actinorrhizal plant species of N.E. India that gradually become endemic with restricted geographic distributions and habitat destruction. Being a natural host of beneficial microbes like endophytes, endomycorrhizae, microfungi including the nitrogen fixing bacterium *Frankia* sp., and *Pseudomonas* sp., etc. this plant harbours potential usages for soil reclamation as well as habitat reconstruction for development of ecological succession.

Keeping the points under consideration, the present investigation has been designed to isolate the hitherto-unexplored endophytic fungal diversity associated with *Elaeagnus latifolia* L.,that could be of key importance in plant protection, drug discovery and other industry related components. Screening for antagonistic potential of the putative endophytic strains against *P. theae*, the causal agent of grey blight disease in tea [*Camellia sinensis* (L.) O. Kuntze]is another objective here to achieve sustainability in tea cultivation as most of the chemicals used to control tea diseases ultimately results in MRL issues in made tea, soil deterioration, environmental pollution, eutrophication of natural water bodies and lethal/sub-lethal effects to non-target organisms including human beings. Isolation and preliminary screening of endophytes against certain tea pathogens like *P. theae*, *Sphereostilbe repens* and *Fusarium solani* has been made by Bora and Barthakur (2013). The workers identified the endophytic isolates based on their colony morphology, spores and fruiting bodies using stereo and light microscopes. Abilities of endophytes to produce secondary metabolites with novel structure create perspective of using such microbial biopesticides in plantation crops like tea. Naturally occurring endophytic strains inhabiting in *E. latifolia* L. may inhibit the growth of target pathogen by hyperparasitic/antagonistic activities. The establishment of large numbers of metabolically active population of endophytic microbes is essential for the success of environmental remediation in agricultural practices. Determination of colonization frequency and diversity indices of endophytic microbes associated with *E. latifolia* L. with putative endophytism is studied, here, that holds perspectives in agriculture and forestry.

Materials and Methods

Collection of the plant specimen and identification

Survey was made at three distinct geographical locations of North-east India such as Jorhat (one of the richest biodiverse zone in Assam with a geographical area of 2859.3 sq. km lies between 26.46 °N latitude and 96.16 °E longitude), Sivasagar (26.45 °N latitude and 95.25 °E longitudes), and Meghalaya (25.65 °N latitude and 91.88 °E longitude) (Fig. 1) for collection of the target plant species, *Elaeagnus latifolia* L., (Fig. 2) which is known for its ability to form symbiotic relationship with several beneficial microbiotas. The collected plant specimens were preserved in herbarium sheet and identified using standard literature.

Fig. 1

Fig. 2

Isolation of endophytic fungi

Pre-sterilized plastic bags were used for collection of plant samples. Excess moisture was removed during sampling. The leaf, root and stem samples were stored at 4 °C for further microbial analysis. Asymptomatic healthy materials were thoroughly washed in tap water, followed by surface sterilization using standard protocol (Petrini 1986). The selected plant segments were immersed in 95% ethanol for 30s, followed by 4% sodium hypochlorite solution for 15s. The segments were then treated with 95% ethanol for 30s. Sterile distilled water (SDW) was used to rinse the segments. The segments were allowed to dry under sterile conditions. Two different protocols were used for isolation of endophytic strains from the target plant species. In one method, leaves, stem and root were cut to uniform sizes (5 mm) and plated on endophytic specific media supplemented with streptomycin (100 mg/L) to suppress the bacterial growth while the other protocol follows plating of ground samples on culture media. Ground sample was mixed with 100 ml SDW to get homogenous suspension and plated accordingly using the serial dilution plate technique. Sabouraud Dextrose Agar (SDA) and Tryptic Soya Agar (TSA) were used as culture media for isolation of endophytic microbes. Petriplates were sealed with parafilm and incubated at 30 ±1°C in BOD incubator for 5-7 days. Regular monitoring of plates is made to observe the growth of endophytic fungal colonies. Fungi grown in the samples were transferred onto fresh potato dextrose agar (PDA) plates and respective broth cultures for isolation of pure colonies.

Identification of endophytic fungal isolates

The morphological characteristics of the fungal isolates were made in accordance with Photita et al. (2005). Ainsworth et al. (1973) was followed to examine and measure the key morphological characters of spores and hyphae including colony diameter, texture, colour, elevations etc., using light and stereo microscopes.

Determination of growth promoting potential

Phosphate solubilization

Phosphate solubilization was detected by the formation of transparent halos surrounding the microbial colonies on Pikovskaya agar medium after 4-5 days of incubation at 30 ±1°C (Pikovskaya 1948).

Starch hydrolysis

For starch hydrolysis test, the organisms were first inoculated on PDA media supplemented with starch and then incubated at 37 ±1°C for 4-6 days. Development of a clear zone around the microbial colonies indicated the hydrolysis of starch.

Determination of Zn solubilization efficiency *in vitro*

Actively growing cultures of each isolated strain were spot-inoculated (approx. 3 μL) onto the agar plates and incubated at 30 ±1°C for 72-96 h. The halo zone around the microbial colony was determined. Repeated streaking on culture media was done for confirmation of the halo zone and determining the zinc solubilizing potential. The diameter of the microbial colony (B) and clear zone around the colony (A) was measured for calculating the solubilization efficiency in percent and area in mm^2. Zn solubilization efficiency was calculated using the following formula:

$$\text{Solubilization efficiency (SI)} = \frac{\text{Solubilization diameter (A)}}{\text{Diameter of the colony growth (B)}} \text{ X } 100$$

Quantitative Analysis

Quantitative analysis of the parameters like density, frequency and abundance of the endophytic populations were determined in accordance with Curtis and McIntosh (1950).

(a) Density

Density is calculated using the following equation

$$\text{Density} = \frac{Total\ number\ of\ individuals\ of a\ species\ in\ all\ compartments}{Total\ number\ of\ compertments\ studied}$$

(b) Frequency (%)

Following formula was used to determine the frequency.

I. Percentage of frequency (%) Compartment-wise:

$$\text{Frequency (\%)} = \frac{Total\ number\ of\ compartments\ in\ which\ the\ species\ occured}{Total\ number\ of\ compertments\ studied} \times 100$$

II. Percentage of frequency (%) Sample-wise:

$$\text{Frequency (\%)} = \frac{Number\ of samples\ in\ which\ a\ particular\ species\ was\ recorded}{Total\ number\ of\ samples} \times 100$$

(c) Abundance

Abundance is represented by the following equation:

$$\text{Abundance} = \frac{Total\ number\ of\ individuals\ of\ a\ species\ in\ all\ compertments}{Number\ of\ compertments\ in\ which\ the\ species\ occured}$$

(d) Percentage of occurrence (%)

The density and distribution of AM fungi in rhizosphere soil was expressed in terms of percentage of occurrence.

$$\text{Percentage of occurrence(\%)} = \frac{Total\ number\ of spores\ of\ individual\ species}{Total\ number\ of\ fungal\ spores} \times 100$$

Determination of colonizing frequency (CF %)

The colonizing frequency (CF %) of endophytic fungal species was calculated using Hata and Futai (1955).

$$\text{colonizing frequency (CF \%)} = \frac{Number\ of\ segments\ colonized\ by\ an\ endophytic\ fungal\ species}{Total\ number\ of\ segments} X\ 100$$

Species richness and diversity indices

Total number of species recovered and endophytic fungal species richness were determined after identifying the fungal spores using following formula:

$$S = n + \left(\frac{n-1}{n}\right) k$$

Where, S = Species richness

n = Total number of species present in the sample population

k = Number of "unique" species

Diversity index

Diversity index was computed using the following formula:

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

Where, D = Diversity Index

N = Total number of organisms of all species found

n = Number of individuals of a particular species

Simpson's index of diversity = 1–D

Where, $D = \in (n / N)^2$

n = Total number of organisms of a particular species.

N = Total number of organisms of all the species.

$$\text{Shannon-Wienner diversity index, } H_s = -\in (Pi)(\ln Pi)$$

$$\text{S}$$

$$\text{i = 1}$$

Where, H_s = Symbol for the diversity in a sample of S species or kind.

S = The number of species in the sample.

Pi = Measures the relative abundance of i^{th} species or kinds = ni/N

N = The total number of individuals of all kinds.

ni = The number of individuals of i^{th} species.

ln = log to base 2.

Antimicrobial bioassay

Preliminary screening of antimicrobial agent producing endophytic microorganisms (AAPEMs) were made using a modified cross streak method as mentioned in Jemimah et al. (2012). For this, a loopfull of microbial inoculums (endophytic fungi) was streaked in the centre of the petridish containing PDA medium and incubated at 28 ±1ºC for 4-5 days. 48-72h old target pathogen (here, *P. theae*, responsible for grey blight disease in tea) was cross inoculated to the growth line of the fungi in the same petriplates at 28 ºC for 48-72 h. The inhibitory zones produced by the endophytic fungus against the target pathogen were measured in millimeters (mm). The pathogen was collected from Microbial Culture Laboratory (MCL), Tocklai Tea Research Institute, TRA, Jorhat, Assam, India. Light to dark brown disease patches in tea leaves with a greyish centre on the upper surface marked with concentric zonations almost from the centre to the very edge in field were selected for pathogen isolation (Fig. 3(a-e). On old leaves, it starts from damage incidences in leaves like cut, break or a bruise on the leaf blade while on young leaves the patch is usually dark brown to almost black rather irregular in shape and not marked with concentric rings.

Fig. 3(a-e)

Poisoned Food technique as described by Balouiri et al. (2016) was followed to assess the antimicrobial activity of the potent strains against *Pestalozzia theae*. For this, the microbial cultures showing antagonistic properties on agar plates was grown on 250 ml PDB medium and incubated in BOD shaking incubator at 28±1 °C up to 15-21 days with periodic shaking at 250 rpm after which the microbial cultures were macerated using waring blender for 10 min. The macerate was then filtered through double-layered muslin cloth and centrifuged properly. Whatman No. 1 filter paper (110 mm) was used to filter the supernatant. The obtained culture filtrate served as the crude extract with 100% spore concentration. Inoculum potential of 5% densities for each microbial culture were prepared using 10^6 spore/ml of the sample and plated accordingly to measure the fungal growth. The final concentration of the desired density (*i.e.,* @5% spore concentration) was incorporated into the molten agar and mixed well. The medium was then poured into petridishes followed by overnight

incubation. Pathogen mycelia disc (5 mm) was then inoculated in the centre of the plate. DMSO served as negative control. The plates were incubated at 28±1°C for 5 days. Plate devoid of antifungal agent served as control. Three replicates were maintained for each treatment. The antifungal efficacy of the biocontrol agent was estimated using the following formula:

$$\text{Antifungal activity (\%)} = \frac{(Dc - Ds)/Dc)}{Dc} X\ 100$$

Here, Dc is the diameter of fungal growth in control plate and Ds is the diameter of fungal growth in the plate containing tested antifungal agent.

Results

During the present investigation, a total of 17 species of fungal endophytes belonging to 12 families and 3 orders were isolated from various parts of *E. latifolia* L as depicted in Table 1. Totally 21 isolates were recovered from 15 samples (5 segments each of stem, roots and 10 disks of leaves).

Table 1

All the taxa were grouped according to their morphological characters. Fig. 4(A-L) shows systematic examination for exploring the endophytic microflora of different parts of the plant *E. latifolia* L. using culture-based approach.

Fig. 4 (A-L)

The growth habit of endophytic microbes on culture plates are shown in Fig 5(a-i).

Fig. 5 (a-i)

The isolated fungal endophytes are identified as *Absidia* sp., *Arthrobotrys* sp., *Aspergillus candidus, Aspergillus* sp., *Basidiobolus* sp., *Chrysosporium* sp., *Cladosporium* sp., *Cunninghamella* sp., *Fusarium* sp., *Mortierella* sp., *Nigrospora* sp., *Paecilomyces* sp., *Penicillium capsulatum, Penicillium chrysogenum, Pythium* sp., *Rhizopus* sp., and *Scopulariopsis* sp. There are also sterile forms recorded based on the culture characteristics and given code numbers. Table 2 represents the diversity analysis of the fungal endophytes isolated from different plant parts of *E. latifolia* L. along with the frequency, abundance and percentage of occurrence. Highest (67%) organ-wise (*i.e.*, leaves, stem and root is indicated as separate organ) isolation frequency was in case of *Fusarium* sp.,

Nigrospora sp., *Penicillium chrysogenum* and *Rhizopus* sp., followed by other species with 33% each. Maximum abundance value (4.0) was observed in *Basidiobolus* sp. followed by *Aspergillus candidus* and *Cunninghamella* sp. (3.0 for each) and lowest (0.5) in *Nigrospora* sp. In contrary to organ-wise frequency, the percent natural occurrence was highest (maximum up to 12%) in case of *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum* and *Rhizopus* sp. (Table 2).

Table 2

The highest density (0.5) was observed in case of *Nigrospora* sp. and lowest (0.13 for each) in *Arthrobotrys* sp., *Aspergillus* sp., *Chrysosporium* sp., *Cladosporium* sp., *Cunninghamella* sp., *Paecilomyces* sp., *Penicillium capsulatum* and *Pythium* sp., respectively (Fig. 6).

Fig. 6

Regarding the occurrence of fungal endophytes in different parts of *E. latifolia* L., it was evident that more fungal isolates were obtained from root (47% colonization frequency) followed by stem (41%) and leaves (29%) respectively as depicted in Fig. 7.

Fig. 7

Isolation frequency of endophytic microbes using different media is depicted in Table 3. The table indicates highest isolation of microbes in TSA media (12.01 cfu/ml) in root and lowest (6.97 cfu/ml) in leaves.

Table 3

Species richness and diversity index showed maximum in stem (15.0); whereas minimum was observed in the leaves (9.0) (Table 4). The highest Shannon and Simpson index value was observed in stem (2.02 and 0.860 respectively); followed by roots (1.979 and 0.847 respectively) and least in leaves with a Shannon diversity index of 1.494 and Simpson diversity index of 0.75 respectively.

Table 4

During the present investigation isolate EF09 (*Fusarium* sp.) showed positive results for different growth promoting parameters like starch hydrolysis, phosphate solubilisation, zinc solubilisation and IAA production

while the rest of the strains are either able to hydrolysis starch, IAA production or solubilise phosphorous or zinc separately (Table 5).

Table 5

The isolates when examined for preliminary antagonistic screening by cross streak method using *P. theae* as target pathogen four isolates, EF03, EF04, EF09 and EF14 showed positive response in growth and pathogen inhibition. Therefore, these microbe were, further, selected for antimicrobial evaluation against *P. theae* using poisoned food technique as mentioned above. The growth habit and morphology of the endophytic isolates selected for antagonistic screening are represented in Fig. 8(A-D). Among the screened endophytic isolates, maximum antifungal potential (%) against *P. theae* was exhibited by EF05 (87.1%) (Fig. 9) followed by EF03 (*Aspergillus candidus*) (43.4%), EF04 (*Aspergillus* sp.) (39.34%) and EF14 (*Penicillium chrysogenum*) (30.3%) respectively.

Fig. 8 (A-D)

Fig. 9

Discussion

The natural resources are being exploited on large scale by human race. Due to over-exploitation of natural forest wealth, some of the forest plant species along with its associated microflora are on the verge of extinction. *E. latifolia* L., due to its diverse applications in agriculture, forestry and medicine needs conservation. *E. latifolia* above and below ground parts posses diverse structural, chemical or biotic mechanisms that may favour for effective colonization by beneficial groups of microbes (Klironomos 2003) including AM colonization and endophytic fungi. Fungal endophytes invade their host through direct penetration, wounds or natural openings (stomata or lenticels). Enumeration of endophytic diversity in different healthy parts such as leaves, stem, fruits and roots of four ethnomedicinal plants i.e. *Digitalis purpurea, Digitalis lanata, Plantago ovata* and *Dioscorea bulbifera* has been made by Ahmed (2012). Caruso et.al. (2000) isolated 150 fungal and 71 actinomycete endophytes from the internal tissues of woody branches, shoots and leaves of different plants of *Taxus baccata* and *Taxus brevifolia*. Arnold et al. (2003) reported that tropical endophytes could be of hyper diverse with host preference and spatial heterogeneity. Pavithra et al. (2020) suggested that the majority of the endophytic fungi are supposed to be ascomycetes and asexual fungi. Furnkranz et al. (2012) made observations on spacial

384 distribution of endophytes in different plant parts. According to Furnkranz et al. (2012) the distribution of
385 endophytes living inside the plants depends strongly on the combination of the allocation of plant resources and
386 the ability to colonize. Endophytes in the roots of plants often penetrate the site at which lateral roots emerge
387 and help in colonizing the epidermis, in the root cracks and below the root hair zone (Zakria et. al. 2007).
388 Colonization of this nature can effectively establish population both intracellularly and intercellularly.

389 Growth promoting potential of endophytic microbial isolates to different plant growth promoting
390 parameters might be attributing their selection for crop improvement programmes. Similar studies on
391 determination of growth promoting potential by endophytic fungi has been made by Nath et al. (2015) and
392 Waqas et al. (2012). Souchie et al. (2006) reported *Aspergillus* sp., and *Penicillium* sp., as efficient phosphate
393 solubilizers. Thongsandee et.al (2012) reported difference in endophytic population and frequency in relation to
394 change in organ-types like young leaves, petiole and twigs of *Gingko biloba* L. According to Giri and Dudeja
395 (2012) plants strictly limit the growth of endophytes and these endophytes use many mechanisms to gradually
396 adapt to their living environments.

397 As one the objective of this study is to evaluate the antifungal activity of endophytic microbial strains
398 isolated from *E. latifolia* L. against *P. theae*, the causal agent of grey blight disease in tea. Organ specific
399 endophyte isolation throughout different parts of the plant is useful here; further, to develop microbial-based
400 bioformulations in tea crop protection. Highest antifungal potential of endophytic isolate, EF09 might be due to
401 its ability to produce secondary metabolites (Mejia et al. 2008) that subsequently works in disease reduction. It
402 is, thus, imperative to explore, select, characterize and manipulate right microorganism to regulate plant
403 pathogens. Isolation, identification and conservation of endemic plant specimens and putative microbial genetic
404 resources in the threatened areas worldwide (Handique et al. 2015) using biological tools and techniques is
405 necessary for *in situ* and *ex situ* conservation of these plant and microbial resources and their natural habitats.
406

407 **Conclusion**

408

409 Although there exists several opportunities in endophytic research, most of the studies about endophytic fungi is
410 still confined on the population biology and restricted to certain plant families viz. Coniferaceae, Ericaceae and
411 Gramineae (Tan and Zou 2001). *Elaeagnus latifolia* L., due to its multiple usage and economical significance is
412 under threat and nearing to its threshold. The study strengthens existing knowledge of fungal endophytes with
413 regards to their occurrence with this actinorhizal plant species. It is apparent that endophytic fungi have

414 profound impacts on the survival and fitness of the plants in all terrestrial ecosystems and therefore likely to
415 play significant role in plant biogeography, evolution and community structure. Isolation of endophytic fungi
416 from *E. latifolia* L. and evaluating its potential against significant tea disease in N.E. India hold perspectives to
417 develop biologically active agents (microbial biopesticides) on commercial scale to sustain global tea research
418 as they can be easily cultured *in vitro* instead of harvesting plants and affecting the environmental biodiversity.
419 Bioprospecting of microbial metabolites for the estimation of bioactive compounds responsible for plant growth
420 promotion and disease control is need of the hour; further, to understand the mechanism of action of potent
421 endophytic microbial populations in relation to disease protection and crop development.
422
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504 **Fig. 1** Map showing the sampling locations (Circles).

505 **Fig. 2** Collection of the plant specimen, *Elaeagnus latifolia* L. for enumeration of endophytic fungal
506 assemblances.

507 **Fig. 3** Field identification of *P. theae* and culture growth pattern on PDA plates.

508 **Fig. 4 (A-L)** Isolation of endophytic microflora from different plant parts of *E. latifolia* L.

509 **Fig. 5 (a-i)** Pure culture plates of certain isolated endophytic strains.

510 **Fig. 6** Density of fungal endophytes isolated from different plant parts of *E. latifolia* L.

511 **Fig. 7** Per cent occurrence of fungal endophytes in *E. latifolia* L.

512 **Fig. 8(A-D)** Morphology of the antagonistic endophytic isolates on PDA media. A; endophytic microbial
513 culture on PDA tubes; B. Broth preparation for antagonistic screening; C. Culture character of isolate, EF09; D.
514 Zinc solubilisation potential by EF09.

515 **Fig. 9** Antimicrobial evaluation of EF09 (*Fusarium* sp.) against *P. theae* using poisoned food method.

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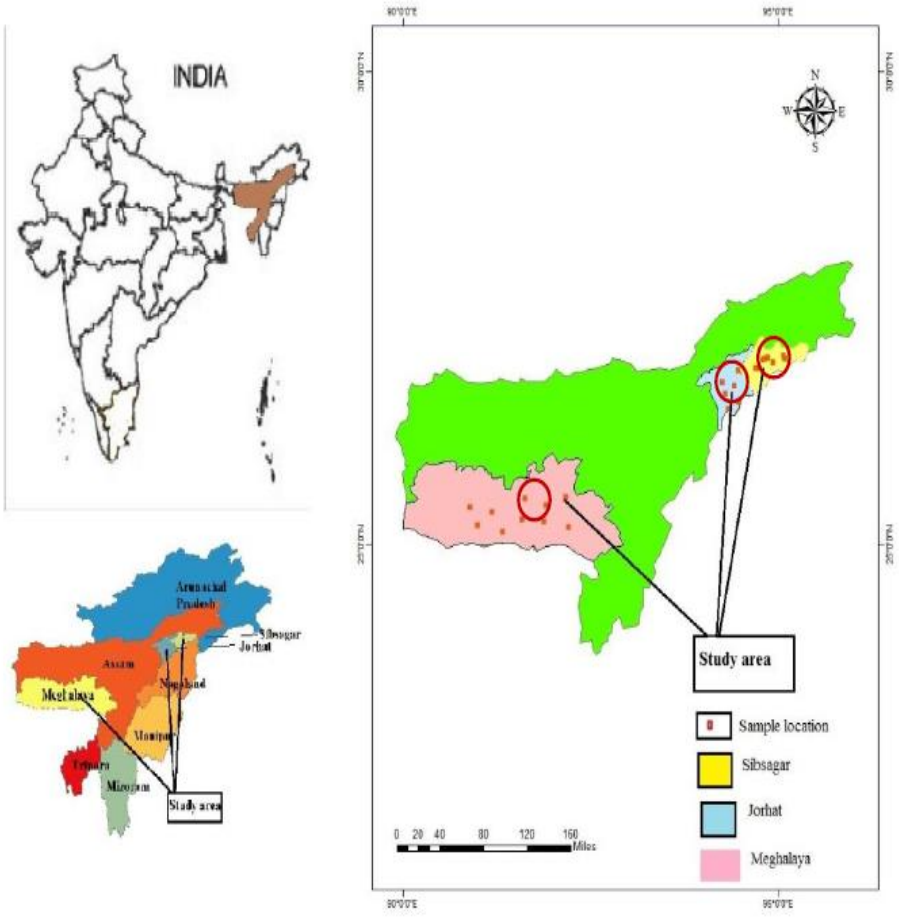

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Fig. 2 Collection of the plant specimen, *Elaeagnus latifolia* L. for enumeration of endophytic fungal assemblances.

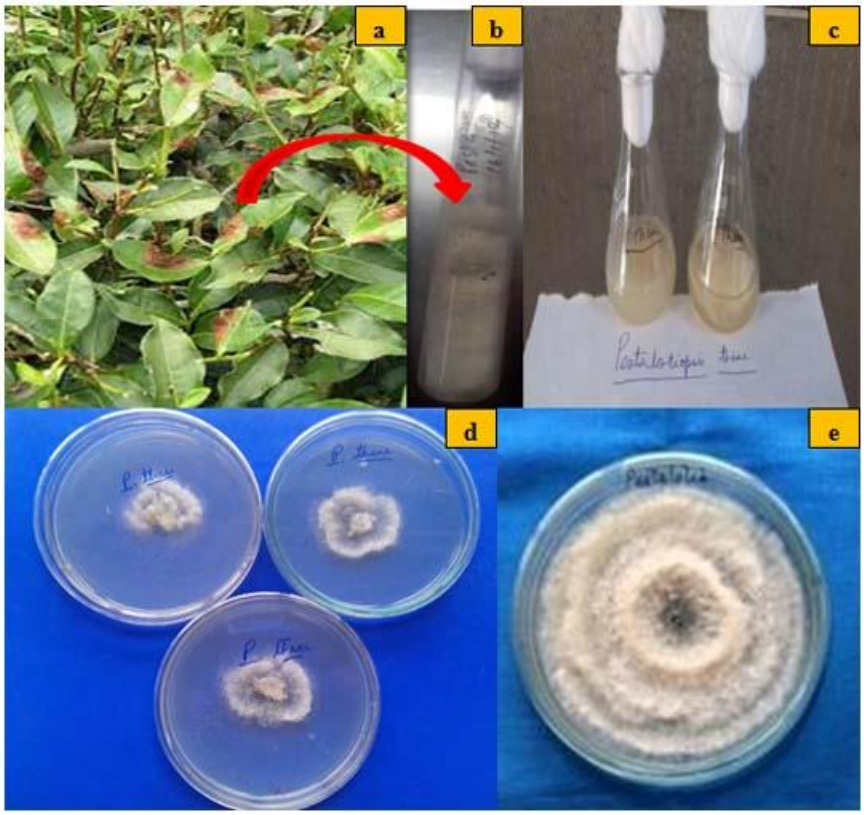
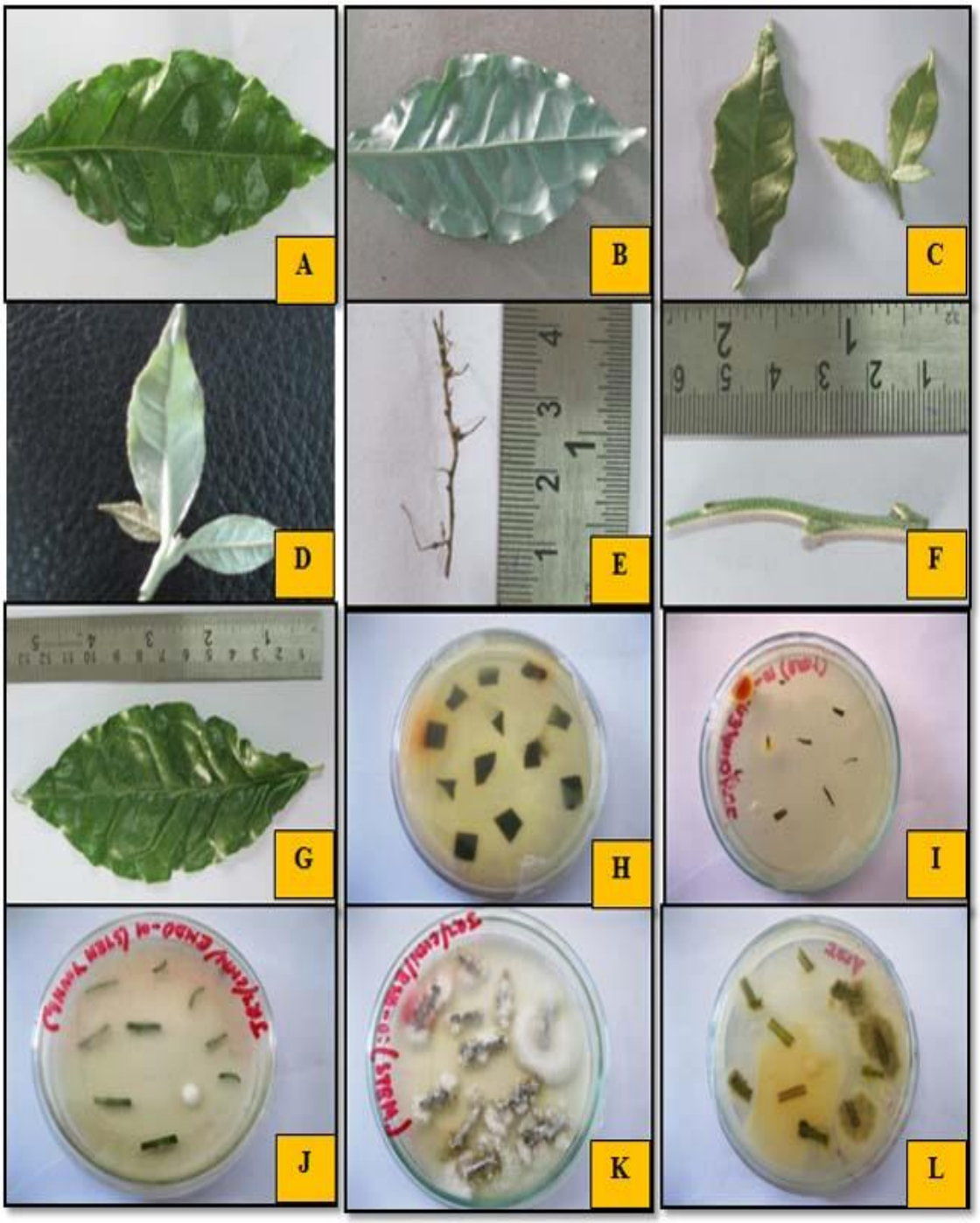


Fig. 3 Field identification of *P. theae* and culture growth pattern on PDA plates.



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562 **Fig. 4 (A-L)** Isolation of endophytic microflora from different plant parts of *E. latifolia* L.

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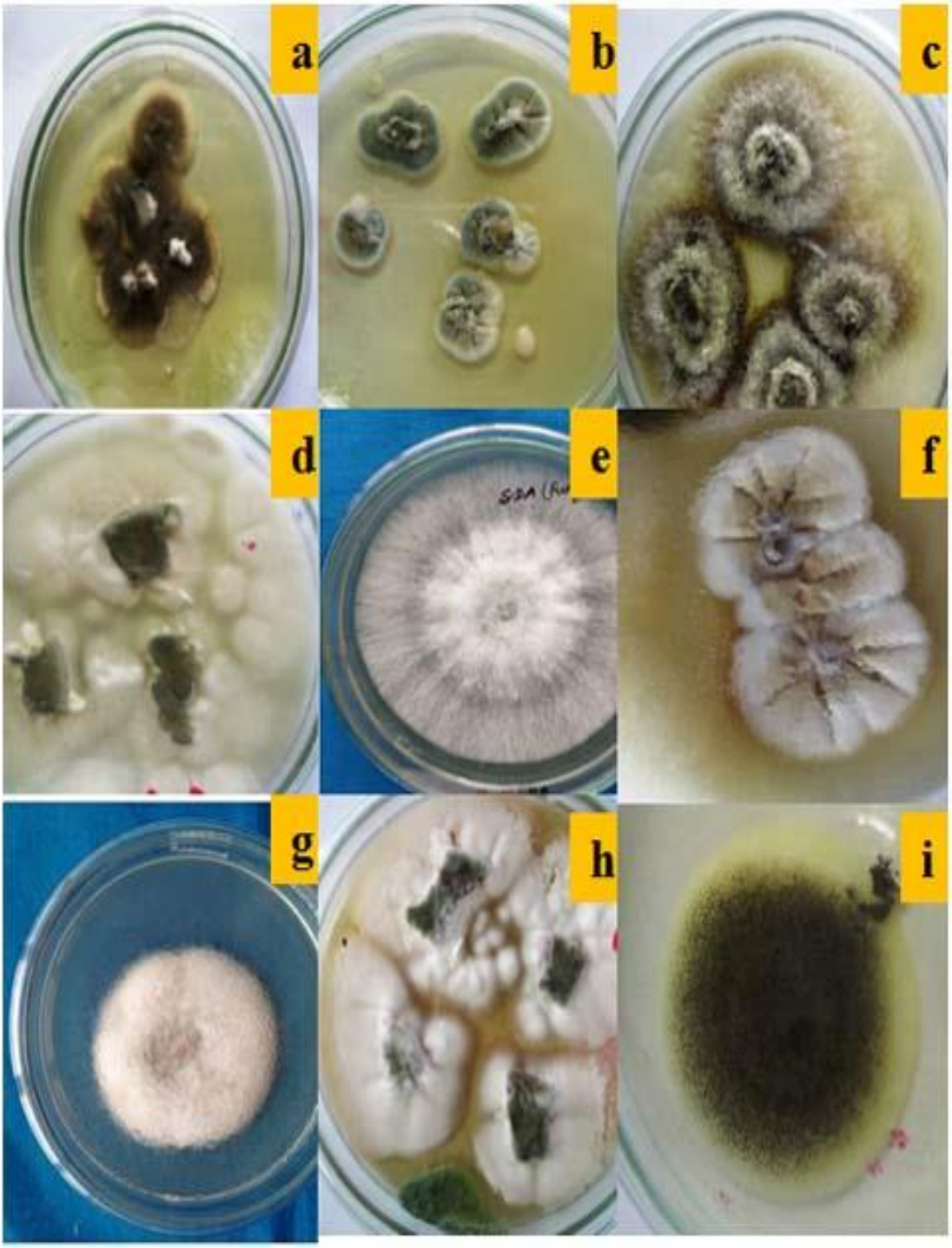
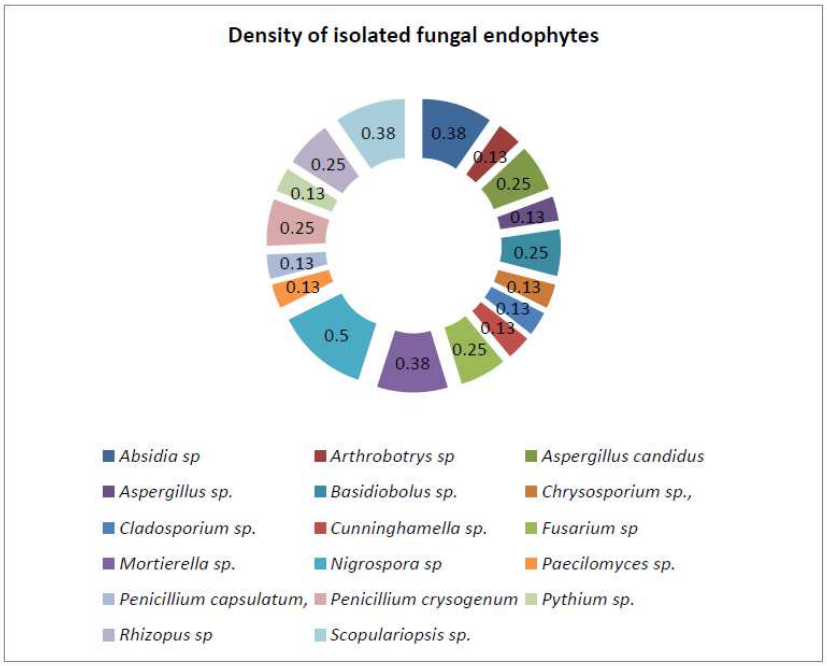


Fig. 5 (a-i) Pure culture plates of certain isolated endophytic strains.



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573 **Fig. 6** Density of fungal endophytes isolated from different plant parts of *E. latifolia* L.

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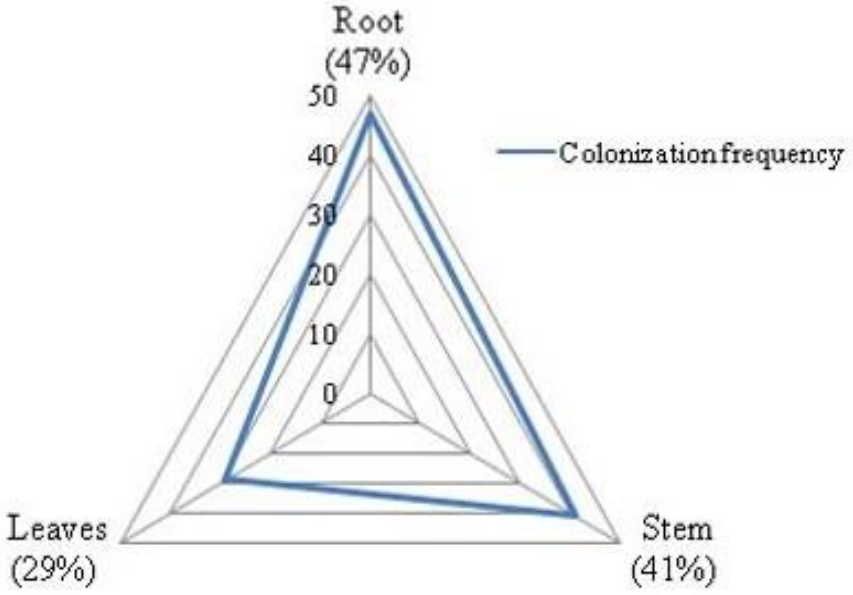

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589 **Fig. 7** Per cent occurrence of fungal endophytes in *E. latifolia* L..

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Fig. 8(A-D) Morphology of the antagonistic endophytic isolates on PDA media. A; endophytic microbial culture on PDA tubes; B. broth preparation for antagonistic screening; C. EF09, culture character; D. Zinc solubilisation potential by EF09.

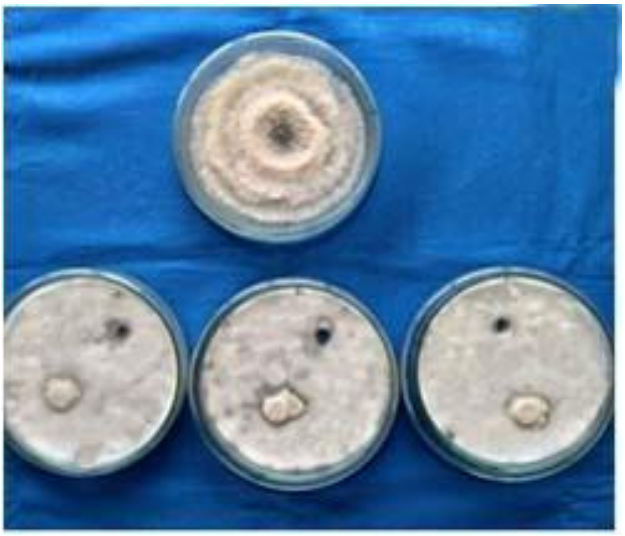

Fig. 9 Antimicrobial evaluation of EF09 (*Fusarium* sp.) against *P. theae* using poisoned food method.

640 **Table 1** Natural occurrence and diversity of endophytes in different plant parts of *E. latifolia* L.

Isolate No.	Name of species	Order	Family	Stem	Leaves	Roots
EF01	*Absidia* sp.	Zygomycota	Mucoraceae	+	-	-
EF02	*Arthrobotrys* sp.	Ascomycota	Orbiliaceae	-	-	+
EF03	*Aspergillus candidus*	Ascomycota	Trichocomaceae	-	+	-
EF04	*Aspergillus* sp.	Ascomycota	Trichocomaceae	-	-	+
EF05	*Basidiobolus* sp.	Zygomycota	Basidiobolaceae	-	-	+
EF06	*Chrysosporium* sp.	Ascomycota	Onygenaceae	-	+	-
EF07	*Cladosporium* sp.	Ascomycota	Davidiellaceae	-	+	-
EF08	*Cunninghamella* sp.	Zygomycota	Cunninghamellaceae	+	-	-
EF09	*Fusarium* sp.	Ascomycota	Nectriaceae	+	-	+
EF10	*Mortierella* sp.	Zygomycota	Mortierellaceae	+	-	-
EF11	*Nigrospora* sp.	Ascomycota	Trichosphaeriaceae	+	+	-
EF12	*Paecilomyces* sp.	Ascomycota	Trichocomaceae	+	-	-
EF13	*Penicillium capsulatum,*	Ascomycota	Trichocomaceae	-	-	+
EF14	*Penicillium chrysogenum*	Ascomycota	Trichocomaceae	-	+	+
EF15	*Pythium* sp.	Oomycota	Pythiaceae	-	-	+
EF16	*Rhizopus* sp.	Zygomycota	Mucoraceae	+	-	+
EF17	*Scopulariopsis* sp.	Ascomycota	Microascaceae	+	-	-

641 "+"= Present, "-" = Absent

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651 **Table 2** Diversity analysis of fungal endophytes in different plant parts of *E. latifolia* L.

Name of the species	Order	Frequency (%)	Abundance	Percentage of occurrence (%)
Absidia sp.	Zygomycota	33	1.0	6.0
Arthrobotrys sp.	Ascomycota	33	1.0	6.0
Aspergillus candidus	Ascomycota	33	3.0	6.0
Aspergillus sp.	Ascomycota	33	1.0	6.0
Basidiobolus sp.	Zygomycota	33	4.0	6.0
Chrysosporium sp.	Ascomycota	33	2.0	6.0
Cladosporium sp.	Ascomycota	33	1.0	6.0
Cunninghamella sp.	Zygomycota	33	3.0	6.0
Fusarium sp.	Ascomycota	67	1.0	12
Mortierella sp.	Zygomycota	33	2.0	6.0
Nigrospora sp.	Ascomycota	67	0.5	12
Paecilomyces sp.	Ascomycota	33	1.0	6.0
Penicillium capsulatum	Ascomycota	33	2.0	6.0
Penicillium chrysogenum	Ascomycota	67	1.5	12
Pythium sp.	Oomycota	33	1.0	6.0
Rhizopus sp.	Zygomycota	67	2.0	12
Scopulariopsis sp.	Ascomycota	33	1.0	6.0

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Table 3 Isolation frequency of endophytes based on source of isolation using different media.

Media types	Source of isolation	Mean (cfu/ml)	Standard Deviation (±)
Tryptic Soya Agar (TSA)	Root	12.01	1.9
	Stem	9.97	0.9
	Leaves	7.12	1.1
Sabouraud Dextrose Agar (SDA)	Root	10.45	1.4
	Stem	8.89	1.1
	Leaves	6.97	1.7

684 **Table 4** Species richness and diversity index of the isolated endophytes.

Source of isolation	Total isolates	Species Richness	Diversity Index	
			Shannon	Simpson
Root	8.0	14.1	1.979	0.847
Stem	8.0	15.0	2.02	0.86
Leaves	5.0	9.0	1.494	0.75

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709 **Table 5** Growth promoting potential of the isolated endophytes.

Isolates	Growth promoting potential			
	Starch hydrolysis	Phosphate solubilization	Zinc solubilization	IAA production
EF01	-	+	+	+
EF02	+	-	-	+
EF03	+	-	+	-
EF04	-	-	+	-
EF05	+	-	-	+
EF06	+	-	+	-
EF07	-	-	+	-
EF08	+	-	+	+
EF09	+	+	+	+
EF10	-	+	+	-
EF11	-	+	-	+
EF12	+	-	+	+
EF13	+	-	+	-
EF14	-	-	-	+
EF15	+	+	-	-
EF16	-	-	-	+
EF17	+	+	-	-

710 '+' Activity showed; '-' Not determined.

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Figures

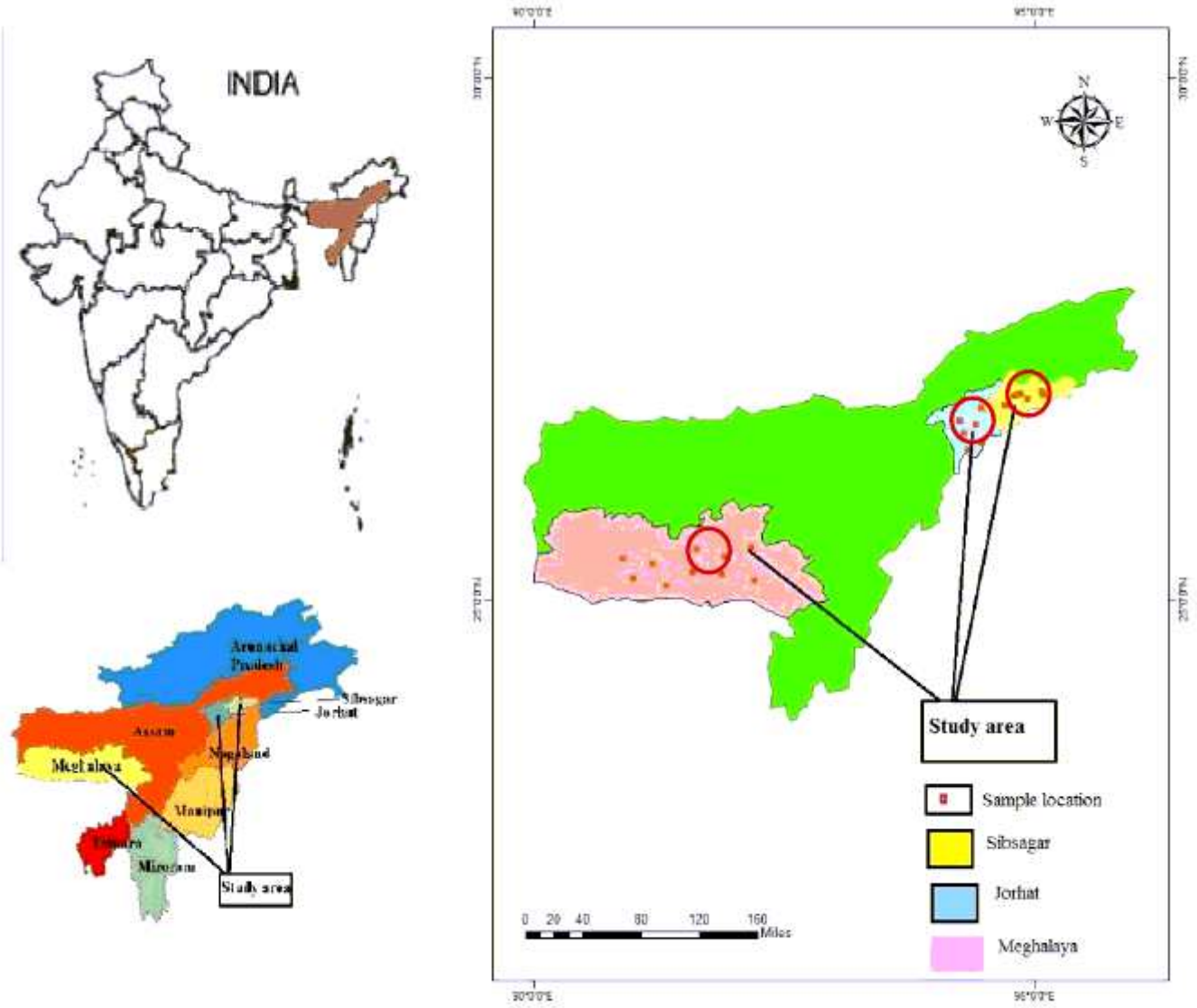

Figure 1

Map showing the sampling locations (Circles). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Collection of the plant specimen, Elaeagnus latifolia L. for enumeration of endophytic fungal assemblances.

Figure 3

Field identification of P. theae and culture growth pattern on PDA plates.

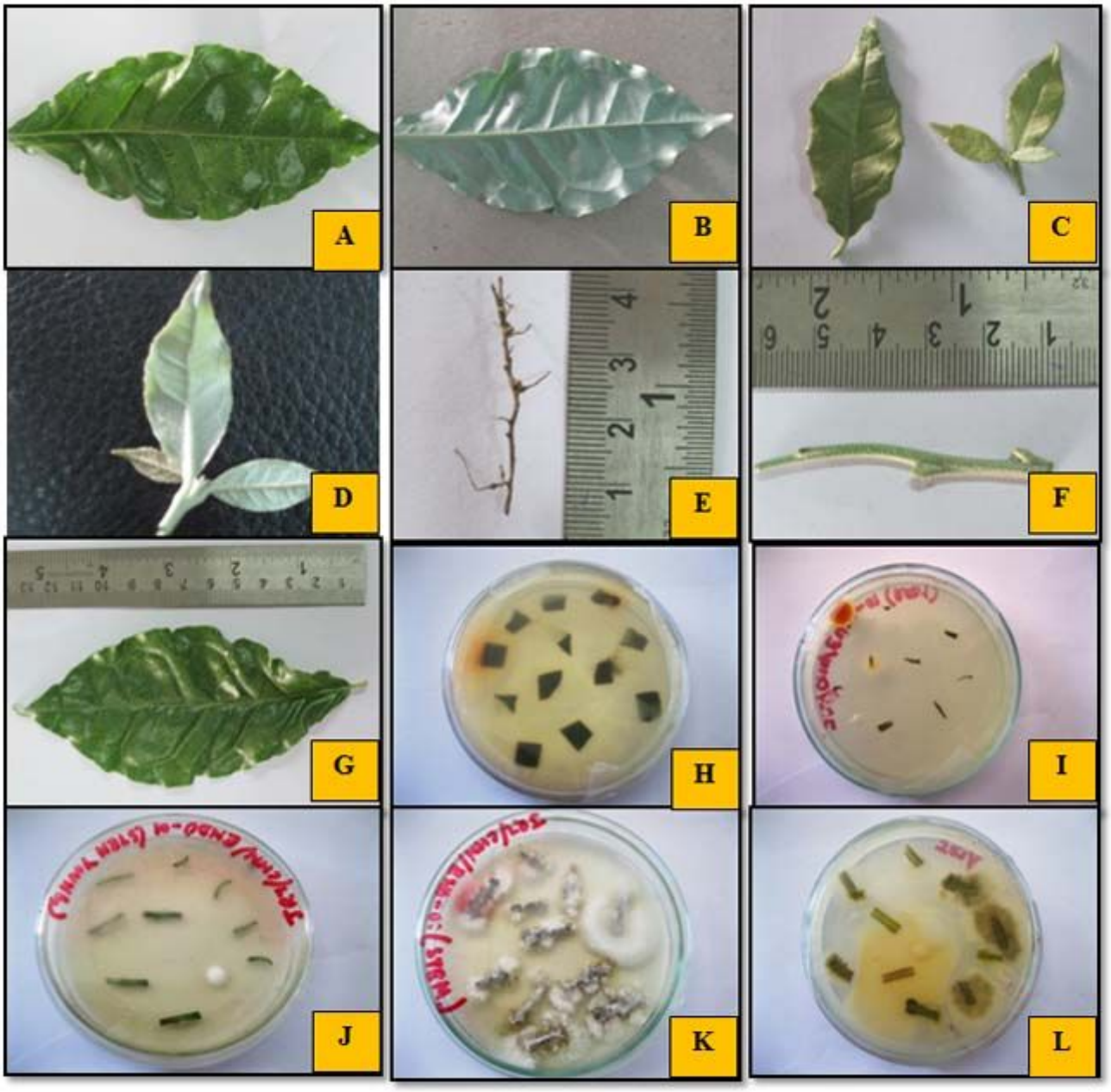

Figure 4

(A-L) Isolation of endophytic microflora from different plant parts of E. latifolia L.

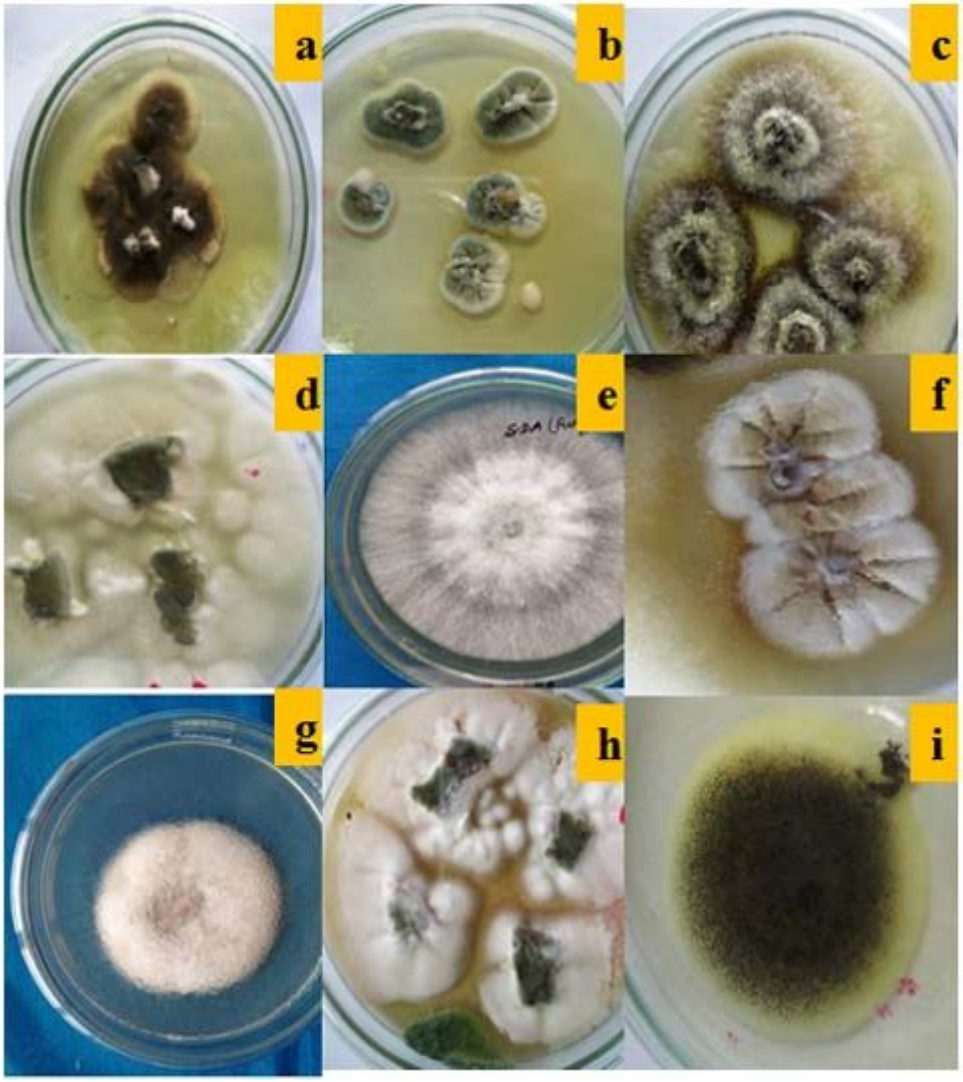

Figure 5

(a-i) Pure culture plates of certain isolated endophytic strains.

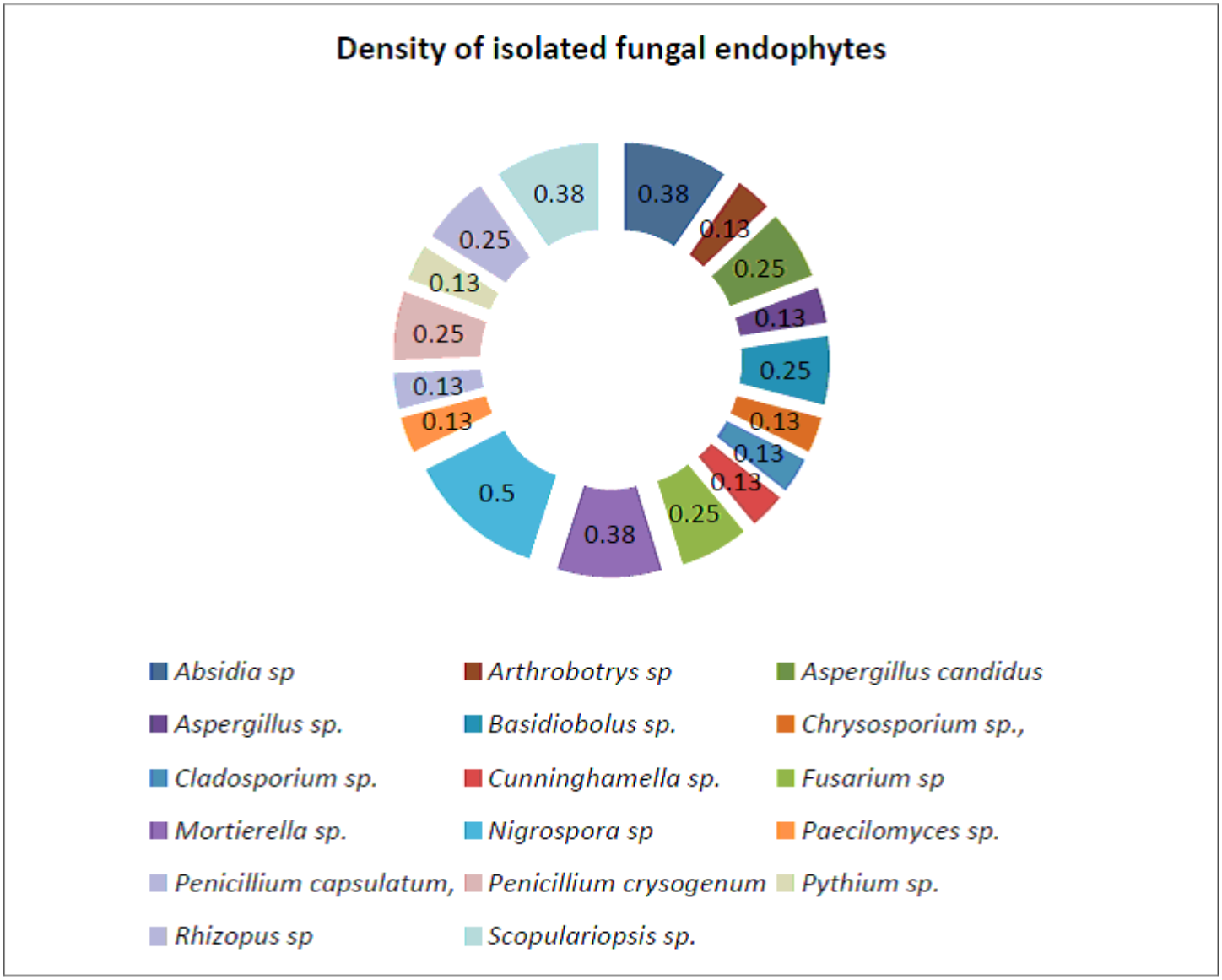

Figure 6

Density of fungal endophytes isolated from different plant parts of E. latifolia L.

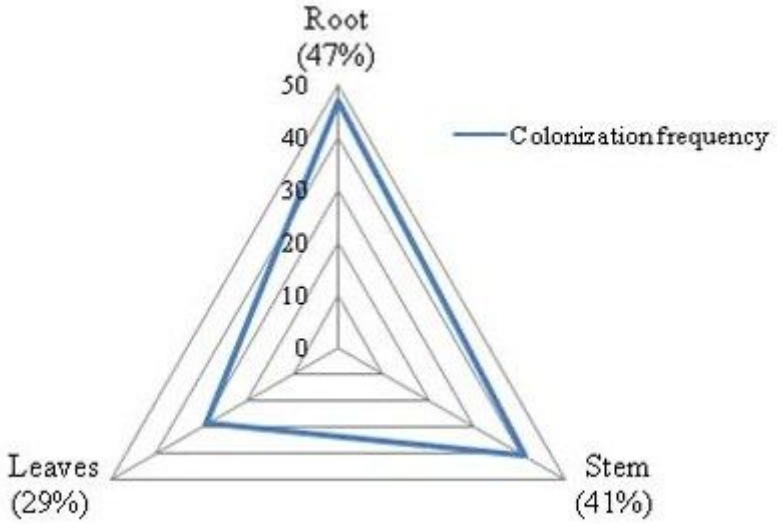

Figure 7

Per cent occurrence of fungal endophytes in E. latifolia L..

Figure 8

(A-D) Morphology of the antagonistic endophytic isolates on PDA media. A; endophytic microbial culture on PDA tubes; B. broth preparation for antagonistic screening; C. EF09, culture character; D. Zinc

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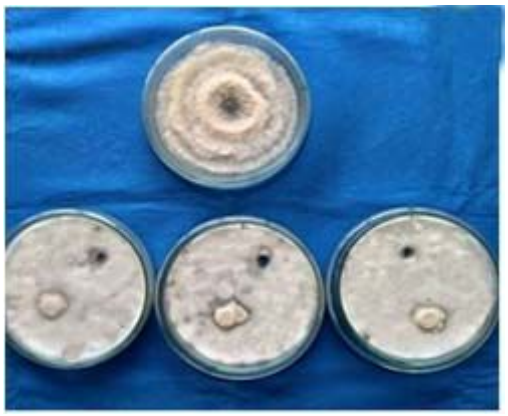

Figure 9

Antimicrobial evaluation of EF09 (Fusarium sp.) against P. theae using poisoned food method.